[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF SASKATCHEWAN]

ACTION OF BENZYLAMINE ON GLUCOSE IN ACETIC ACID SOLUTION. III

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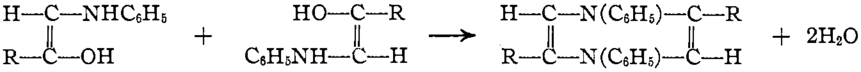
The reaction between amines and glucose is being studied in the hope that it may throw some light on the reaction of amino acids and carbohydrates. Not only do aromatic and aliphatic amines react with carbohydrates, but amino acids do also, and apparently in a similar manner. Thus Irvine and his collaborators have shown that alanine[1] and *o*-aminobenzoic acid[2] react with glucose in the cold just as aniline does. However, when *o*-aminobenzoic acid is heated with glucose, extensive decomposition occurs and little of the *o*-carboxy-anilide can be isolated. Similarly, it has been shown that when glucose and glycine[3] are heated in water solution, cyclic condensation products containing nitrogen are formed. Again, it is found that there is the greatest increase of "humin" nitrogen from dextrose and protein hydrolysates[4] when the solution is neutral or weakly acid. This shows that glucose may react directly with the amino acids and that this compound may give rise to the humins.

When glucose is allowed to react with amines such as aniline[5] in the presence of acetic acid, apparently the same type of reaction occurs. First a compound, glucose-anilide, is formed and this in the presence of acid undergoes further changes; glucose-anilide disappears and brown condensation products are formed.

The latter change is still the subject of study, but tentatively the following explanation is offered. It has already been indicated that glucose-anilide in acid solution becomes "reactive." This might be due to the formation of the aldehyde isomer, which then may change to the enol or break down to methyl glyoxal. If the enol does form, then there may be formed nitrogen ring compounds of various types of which the formation of 2,5-ditetroxybutyl-1,4-diphenyl pyrazine is an example.



$$\begin{array}{l} H\text{—}C\text{—}NHC_6H_5 \\ \quad\| \\ R\text{—}C\text{—}OH \end{array} + \begin{array}{r} HO\text{—}C\text{—}R \\ \|\quad\;\; \\ C_6H_5NH\text{—}C\text{—}H \end{array} \longrightarrow \begin{array}{l} H\text{—}C\text{—}N(C_6H_5)\text{—}C\text{—}R \\ \quad\| \qquad\qquad\qquad \| \\ R\text{—}C\text{—}N(C_6H_5)\text{—}C\text{—}H \end{array} + 2H_2O$$

Where R = $CH_2.OH.(CHOH)_3$

This would be similar to the formation of 2,5-ditetroxybutyl pyrazine[6] from fructose and ammonia in methyl alcohol solution.

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[1] Irvine and Hynd, *J. Chem. Soc.*, **99**, 161 (1911).

[2] Irvine and Gilmour, *ibid.*, **95**, 1545 (1909).

[3] Maillard, *Compt. rend.*, **154**, 66 (1912).

[4] Dowell and Menaul, *J. Biol. Chem.*, **40**, 131 (1919).

[5] Cameron, THIS JOURNAL, **48**, 2233, 2737 (1926).

[6] Stolte, *C. A.*, **2**, 1978 (1908).

On the other hand, it was noticed that glucose-benzylamide residues in methyl alcohol containing acetic acid which had been left exposed to the air became colored, and a distinctly quinoline-like odor developed. Hence it was thought that methyl glyoxal or pyruvic acid might be formed from the "reactive" glucose and that one of these and the amine gave rise to the colored product. This would be comparable to the formation of pyrazine derivatives from ammonia and phenyl glyoxal,[7] or to the production of methyl cinchoninic acid from aniline and pyruvic acid.[8]

It has been found, in fact, that alcoholic solutions of glucose-benzylamide containing acetic acid, in 24 hours gave a test for methyl glyoxal;[9] also, that solutions of benzylamine, glucose and acetic acid in alcohol soon give the same test, the time varying with the concentration of the reactants. Solutions of benzylamine and glucose did not give the test during the time of observation. As a test which depends upon a change of color of sodium nitroprusside in alkali and in acid is liable to be misleading, an attempt was made to isolate the methyl gloxal as the osazone. It was found that by adding *p*-nitrophenylhydrazine to solutions of glucose, benzylamine and acetic acid, the corresponding osazone of methyl glyoxal[10] slowly forms and can be identified. Similarly, from acid solutions of glucose-benzylamide in the presence of *p*-nitrophenylhydrazine, the same osazone is formed in small amounts. Apparently, then, one of the products of the action of benzylamine and glucose in the presence of acetic acid is methyl glyoxal and this, of course, may react with the benzylamine to give various nitrogen ring compounds, but whether this is the only mechanism is still an open question.

Experimental Part

Preparation of Glucose-benzylamide.—Ten g. of glucose was dissolved in 200 cc. of hot 95% alcohol and 17.8 g. (3 molecular proportions) of benzylamine added. The reaction mixture was heated for ten minutes at boiling; it was filtered hot and allowed to stand for 48 hours. It was then cooled in ice water and a gelatinous precipitate separated. This was removed by filtration, thoroughly washed with anhydrous ether and dried in a vacuum. This method yielded a slightly yellow substance, m. p. 70–75°, the yields varying from 80–85%. A 3% solution in methyl alcohol gave $\alpha = -0.61$ to $-0.52°$ in 24 hours in a 1-dcm. tube.

Glucose-benzylamide, recrystallized four times from methyl alcohol and anhydrous ether, is a colorless substance with a bitter taste. It is somewhat soluble in water, but solution is slow. Rather easily hydrolyzed, it reduces Fehling's solution on heating. It is quite soluble in cold methyl and in hot ethyl alcohol, but from the latter it separates as a gel on cooling; it is only slightly soluble in ligroin, benzene, ether and ethyl acetate. The pure material melted at 81.5°; a 3% solution in methyl alcohol gave $\alpha = -1.28$ to $-0.68°$ in 24 hours in a 1-dcm. tube, whence $[\alpha]_D^{22} = -22.66°$.

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[7] Pinner, *Ber.*, **38**, 1531 (1905). See also Gastaldi, *C. A.* **16**, 112 (1922).

[8] Simon, *Ann. chim. phys.*, [7] **19**, 433 (1896).

[9] Neuberg, *Biochem. Z.*, **71**, 150 (1915).

[10] Dakin and Dudley, *J. Biol. Chem.*, **15**, 130 (1913).

Anal. Calcd. for $C_{13}H_{19}O_6N$: C, 57.96; H, 7.12; N, 5.20. Found: C, 57.92; H, 7.19; N, 5.24.

Glucose, Benzylamine and Acetic Acid.—When glucose is allowed to react with benzylamine in ethyl alcohol at room temperature and the reaction is followed by means of the polariscope, it is found (Table I) that during the first six hours the rotation becomes very slightly more positive, then gradually less positive and finally negative. That is, the reaction is of the same type as those previously studied by Irvine.[11] When acetic acid is also present, the preliminary lag in the rotation is not apparent; the rotation gradually became negative just as was the case with aniline, glucose and acetic acid.

TABLE I

EFFECT OF ACETIC ACID ON THE REACTION BETWEEN GLUCOSE AND BENZYLAMINE

Glucose, 1.5 g.; ethyl alcohol to 50 cc.; tube, 1 dcm.; temp., 22–24°.

Benzylamine	3 mole propn.		6 mole propn.	
acetic acid	None	3 mole propn.	None	6 mole propn.
Time	α	α	α	α
5 min.	+1.72	+1.28	+1.74	+0.30
1 hour	+1.72	+0.42	+1.73	− .04
6 hours	+1.76	+ .08[a]	+1.79	− .27[a]
1 day	+1.28	− .32	+1.26	− .92
3 days	−0.50	− .88	−0.52	Red
4 days	− .82	Red	− .89	
8 days	−1.16[a]		−1.18[a]	

[a] First tinge of yellow noted.

Effect of Changing the Concentration of Acetic Acid on the Reaction between Glucose and Benzylamine.—As glucose solutions containing benzylamine and acetic acid in a 1:1 proportion were acid to litmus five minutes after mixing, it seemed worth while to study the reaction when the ratio of amine to acid was in other proportions, in an attempt to keep the reaction near the neutral point. The result is shown in Table II.

TABLE II

EFFECT OF CHANGE OF CONCENTRATION OF ACETIC ACID ON THE REACTION BETWEEN BENZYLAMINE AND GLUCOSE

Glucose, 1.5 g.; benzylamine, 0.89 g. (mole-propn.); alcohol to 50 cc.; tube, 1 dcm.; temp., 23–24°.

Acetic acid Time	None (1) α	0.5 mole propn. (2) α	0.75 mole propn. (3) α	1 mole propn. (4) α
5 min.	+1.73	+1.70	+1.68	+1.66
15 min.	+1.74	+1.64	+1.53	+1.48
30 min.	+1.73	+1.53	+1.37	+1.28
1 hour	+1.73	+1.39	+1.06	+1.08
2 hours	+1.74	+0.96	+0.73	+0.93
3 hours	+1.76	+ .63	+ .62	+ .88
6 hours	+1.76	+ .27	+ .57	+ .80
1 day	+1.45	+ .12	+ .33	+ .52
4 days	−0.08	− .27	− .28	− .22
5 days	− .07	Yellow	Orange	Orange-red

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[11] (a) Irvine and Gilmour, *J. Chem. Soc.*, **93**, 1429 (1908). (b) Irvine and McNicoll, *ibid.*, **97**, 1449 (1910).

The hydrogen-ion concentration was followed approximately by means of indicators. Reaction mixture No. 1 was alkaline to phenolphthalein during 20 days. No. 2 was alkaline on mixing, but gradually the alkalinity diminished until in six hours phenolphthalein was not affected and in two days it became slightly acid to litmus and remained thus for 20 days. On mixing, No. 3 was slightly alkaline, but in 30 minutes it had no effect on phenolphthalein; in three hours it was slightly acid to litmus and remained acid. The fourth solution became faintly acid to litmus 15 minutes after mixing and remained acid thereafter.

It might be pointed out, then, that Reaction 2 (Table II) took place, colored material was produced, it gave the test for methyl glyoxal described later and yet the hydrogen-ion concentration at all times was not far from the neutral point. Curiously also this reaction, slower at first, was most rapid as measured by the change in rotation during a 24-hour period.

Glucose-benzylamide and Acetic Acid.—The colored material produced in glucose, benzylamine and acetic acid solutions is due first (a) to the formation of glucose-benzylamide and then (b) to the action of acetic acid on this compound as indicated by the following.

(a) To an alcohol solution of 3 g. of glucose were added 5.35 g. of benzylamine and 3 g. of acetic acid and the solution was made up to 50 cc. After three days, a large excess of anhydrous ether was added to the ice-cooled reaction mixture and the gelatinous precipitate which separated was removed by filtration. This material was glucose-benzylamide; yield, 0.55 g.; m. p., 80–81°; 0.3422 g. in 25 cc. of methyl alcohol gave $\alpha = -0.30°$ in a 1-dcm. tube, hence $[\alpha]_D^{22} = -21.9°$.

(b) It was found that a 3% solution of glucose-benzylamide in methyl alcohol, in a 1-dcm. tube, gave $\alpha = -1.27°$ in five minutes, —0.69° in 24 hours and —0.64° in 24 days. The solution was colored only a light yellow in 40 days. A comparable solution, containing 0.67 g. of acetic acid in 100 cc. of a 3% solution of glucose-benzylamide in methyl alcohol gave, in a 1-dcm. tube, $\alpha = -0.69°$ in five minutes and —0.42° in six hours. It became light yellow in one day and was red-brown in seven days.

Test for Methyl Glyoxal.—It was found that alcohol solutions of glucose-benzylamide, containing acetic acid and solutions of glucose, benzylamine and acetic acid gave the following test for methyl glyoxal, whereas comparable blanks did not.

To 0.5 cc. of the solution, suspected to contain methyl glyoxal, was added 0.5 cc. of freshly prepared solution of 2% sodium nitroprusside. On the addition of 0.5 cc. of 10% sodium hydroxide the mixture turns red if methyl glyoxal is present. When the reaction mixture is made acid with acetic acid, a violet color is produced. This color, as a rule, persists for some time.

When the test was applied to 3% alcohol solutions of glucose, fructose, glucose-benzylamide, glucose and benzylamine (1, 3 and 6 molecular proportions) during a period of 24 days, the alkaline mixture was yellow; on acidifying with acetic acid, an amber color developed. On the other hand, a 3% solution of glucose-benzylamide in alcohol, containing 0.67 g. of acetic acid (1 molecular proportion) gave a slight test in six hours, a positive test in one day and continued to give it for at least 30 days.

Similarly, alcohol solutions of glucose (3%), benzylamine and acetic acid (1, 3 and 6 molecular proportions, as in Table I) gave a positive test for methyl glyoxal in one day after mixing, and continued positive.

p-Nitrophenylosazone of Methyl Glyoxal.—To 100 cc. of a 48-hour old solution of 3% glucose containing benzylamine and acetic acid (3 molecular proportions), were added 1 g. of *p*-nitrophenylhydrazine and 0.35 cc. of glacial acetic acid. The reaction mixture was shaken until the phenylhydrazine had dissolved and was then filtered from a slight residue. An amorphous precipitate slowly formed and in six days this was removed. The amount was so small that nothing definite could be decided. A further 1 g. of *p*-nitrophenylhydrazine was added, and 40 days after the first addition of phenylhydrazine the precipitate was removed. The precipitate was washed with hot alcohol and dried in a vacuum. It weighed 0.18 g.; m. p., 260–269°, with decomposition. It gave the test for the *p*-nitrophenylosazone of methyl glyoxal.[10] The precipitate was washed with hot 10% sodium carbonate solution, then with water, alcohol and ether. The alkaline filtrate gave no precipitate on acidifying with acetic acid, showing the absence of the *p*-nitrophenylhydrazone of pyruvic acid. The original precipitate was recrystallized from nitrobenzene and toluene; the crimson needles obtained weighed 0.10 g.; m. p., 299–300°. When mixed with the *p*-nitrophenylosazone of methyl glyoxal, the melting point was unchanged. Other samples of a similar material were obtained and analyzed.

Anal. Calcd. for $C_{15}H_{14}N_6O_4$: N, 24.56. Found: 24.62.

In acid solutions of glucose-benzylamide containing *p*-nitrophenylhydrazine, the amorphous precipitate forms only very slowly. Thus in 40 days only 0.08 g. of material was obtained from 100 cc. of a methyl alcohol solution of 3 g. of glucose-benzylamide, 2 g. of *p*-nitrophenylhydrazine and 1.8 g. of acetic acid. This precipitate, when purified, melted at 297–298° and gave the color test for the *p*-nitrophenylosazone of methyl glyoxal.

Glucose-Benzylamide in Water.—It has been found that with glucose, aniline and acetic acid or with glucose-anilide and acetic acid in water solution, a brown precipitate gradually forms and it was thought that glucose-benzylamide would react in a similar manner. Apparently, however, this substance in water hydrolyzes directly to glucose and benzylamine (Table III); the solutions do not yield colored products nor do they give the test for methyl glyoxal.

TABLE III

GLUCOSE-BENZYLAMIDE IN WATER

Glucose-benzylamide, 0.5 g.; water to 50 cc.; tube, 1 dcm.; temp., 20–22°.

Acetic acid Time[a]	None α	0.11 g. (1 mole propn.) α
5 min.	−0.43	+0.10
15 min.	− .42	+ .31
30 min.	− .42	+ .33
1 hour	− .40	+ .34
1 day	− .30	+ .34
2 days	− .23	+ .35
24 days	+ .14	

[a] Time was computed from contact of the glucose-benzylamide and water, but with acid present from the time of addition of the acid, five minutes after solution.

Glucose-benzylamide in water slowly hydrolyzed until in 36 days the solution became opalescent due to the separation of benzylamine. During 80 days, no coloration was noted and no test for methyl glyoxal was obtained. With 1 molecular proportion of acetic acid present the glucose-benzylamide quickly hydrolyzed, so that in 30 minutes it was practically complete. (Complete hydrolysis of 0.5 g. of glucose-benzylamide should give a rotation of +0.35°.) In 80 days, no color developed and the methyl glyoxal test was negative. Even with a small concentration of acid present, as in the experiment below, in 80 days there was no colored precipitate and no test for methyl glyoxal but the reaction mixture had a slight quinoline-like odor.

To 0.5 g. of glucose-benzylamide in water, 0.028 g. of acetic acid (0.25 molecular proportion) was added and the solution made up to 50 cc. Five minutes after the addition of acid and 15 minutes after contact of the amide and water, the rotation in a 1-dcm. tube was —0.28°, in six days it was —0.08° and in 24 days, —0.20°.

Glucose, Benzylamine and Acetic Acid in Water.—The following experiments indicate that benzylamine and glucose in water react to form glucose-benzylamide. However, with 1 molecular proportion of acetic acid present, no reaction apparently occurs, which would be expected from the action of acid on glucose-benzylamide in water. Apparently acetic acid catalyzes the hydrolysis of glucose-benzylamide to a greater extent than it does the "activation." Then one would expect that with a low concentration of acid, activation might occur and brown products form. This is found to be true.

To a water solution of 1.5 g. of glucose was added 0.89 g. of benzylamine (1 molecular proportion) and 0.5 g. of acetic acid (1 molecular proportion) and the solution was made up to 50 cc. During six days (at 20–21°) there was no change in rotation and the test for methyl glyoxal was negative. In 80 days, no colored material had formed.

To a water solution of 1.5 g. of glucose was added 0.45 g. of benzylamine (0.5 molecular proportion) and the solution was made up to 50 cc. In six days (at 20–21°) the rotation in a 1-dcm. tube had changed from −1.51 to −0.40°, but thereafter the reaction mixture became opalescent due to the separation of a small amount of glucose-benzylamide. During 20 days it gave no test for methyl glyoxal and in 80 days no colored precipitate.

A similar glucose solution, containing 0.89 g. of benzylamine (1 molecular proportion) and 0.06 g. of acetic acid (0.0125 molecular proportion) in 50 cc., was cloudy from the start and the rotation could not be read. During 20 days the test for methyl glyoxal was negative but gradually a small amount of brown oil collected and the solution had a nicotine-like odor. After 80 days (at 20–21°) the oil was extracted with ether, the ether evaporated and the residual oil fused with potassium hydroxide, when a quinoline-like odor was detected. However, the amount of material was too small to ensure any definite conclusions as to its nature.

Summary

It is shown that in alcohol, benzylamine and glucose in the presence of acetic acid react to form glucose-benzylamide and that the latter is then, in part at least, converted into methyl glyoxal. The methyl glyoxal, presumably, reacts with the amine to form nitrogen ring compounds It is found that the acetic acid catalyzes the reaction between benzylamine and glucose and that even small amounts of acid are effective,

so that the production of the methyl glyoxal may occur in reaction mixtures which are near the neutral point.

In water solutions, glucose does not react with benzylamine in the presence of acetic acid, when the amine and acid are in a one to one ratio but when the amount of acid used is very small, then reaction does occur as in alcohol. Glucose-benzylamide in water solution is hydrolyzed very readily to the constituent compounds, and the formation of methyl glyoxal or nitrogen ring compounds was not apparent under these conditions.

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THE MECHANISM OF THE CHANGE OF ISONITRO TO NITRO COMPOUNDS

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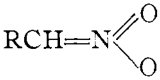
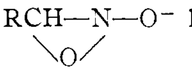
Introduction

It has been shown by Hantzsch[1] that the reversible electrolytic dissociations of nitromethanes are slow enough in both directions to permit the measurement of the rates of change of electrical conductivity when nitromethanes react with bases to form salts or when nitromethanes are formed from their salts by reaction with hydrogen ion. He explained the anomalously slow rates of these homogeneous ionic reactions as due to accompanying changes of structure. The formula RCH_2NO_2 has been accepted as that of the nitromethanes, while the alternative formulas  $\mathrm{RCH{=}N}\begin{array}{l}\nearrow\!\!\!\nearrow \mathrm{O}\\ \searrow \mathrm{O}^-\end{array}$ and  $\mathrm{R}\underset{\diagdown\mathrm{O}\diagup}{\mathrm{CH{—}N}}\mathrm{{—}O^-}$ have been assigned to the ions. Hantzsch prefers the latter formula. This theory is supported by the existence of an isomeric but unstable form of phenylnitromethane, which can be obtained from solutions of the salts of phenylnitromethane by rapid precipitation with hydrochloric acid.

Hantzsch's measurements of the rates of these reactions are not sufficiently accurate to show the mechanisms of the changes, although they do indicate that these are somewhat complex. We have, therefore, attempted to elucidate the mechanisms of these reactions through more accurate measurements of the rates.

Phenylnitromethane and nitromethane were chosen as examples of aryl-substituted and purely aliphatic nitromethanes, respectively, both of these substances having previously been used by Hantzsch. *p*-Nitrophenol was chosen as an example of a different type of nitro compound

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[1] Hantzsch, *Ber.*, **29**, 2251 (1896); **32**, 607 (1899).